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Research Note

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Simultaneous Determination of Morphine HCl, Ketamine HCl and Droperidol in 0.9% Sodium Chloride by HPLC

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ABSTRACT

A simple and rapid liquid chromatographic method is presented for the determination of morphine HCl, ketamine HCl and droperidol in 0.9% sodium chloride injection stored in polyvinyl chloride (PVC) infusion bags. Assay was performed on a pre-packed HPLC Hypersil BDS C18 3.9 mm × 15 cm, particle size 3 µm column under controlled ambient temperature. Separation among morphine HCl, ketamine HCl, droperidol and their associated degradation compounds was achieved by isocratic elution using mobile phase consisting of methanol/acetonitrile/0.01 M 1-octane sulfonic acid sodium in 0.005 M phosphate buffer (pH 4.0) (50/50/100) at a flow rate of 1.0 ml/min. An UV/VIS variable programmable wavelength detector set at 254 nm was used. Sample volumes of 20 µL were injected. There was no need for sample pre-treatment.

Key words: morphine HCl, ketamine HCl, droperidol, 0.9% sodium chloride, HPLC

INTRODUCTION

Morphine HCl is a potent opioid analgesic[(1)]. On the other hand, droperidol is a potent butyrophenone neuroleptic that is commonly used with fentanyl citrate in combination product to effect neuroleptananalgesia[(2)]. Ketamine HCl is used as an anesthestic for diagnostic or short surgical operations[(3)]. A solution of 1.0 mg/mL morphine HCl, 1.0 mg/mL ketamine HCl and 21.4 µg/mL droperidol in 0.9% sodium chloride is commonly used in the treatment of cancer patients. Individual analyses of morphine HCl, ketamine HCl and droperidol have been performed utilizing titration[(4)], spectrophotometry[(5)] and HPLC[(6~12)]. However, a single method for the simultaneous determination of these three active ingredients in 0.9% sodium chloride is still lacking. The objective of this study was to develop a rapid and precise stability-indicating HPLC method for the simultaneous determinations of morphine HCl, ketamine HCl and droperidol in 0.9% sodium chloride solution.

MATERIALS AND METHODS

I. *Materials*

The following injection solutions were used: morphine HCl injection, 10 mg/mL, 1.0 mL, National Bureau of Controlled Drugs, Executive Yuan, Taiwan, ROC (lot 890816); ketamine HCl (Ketalar®), 50 mg/mL, 10 mL. Pake-David, USA (lot ZH708); droperidol (Dehyobenzperidol®), 2.5 mg/mL, 10 mL, Janssen Pharmaceutic, USA (lot 00EB314); 0.9% sodium chloride injection, 100 mL,Y. H. Pharma., Taiwan, ROC (lot 107A01E). The primary reference standard of ketamine HCl (lot 788003) was a gift from Pake-David Pharmapeutic, Australia. The primary reference standard of morphine HCl (NM410-871001) was from National Bureau of Controlled Drugs, Executive Yuan, Taiwan, ROC and droperidol (lot 9013-1) was provided by Jannsen Pharmaceutic, USA. 1-Octane sulfonic acid sodium was from Fisher Scientific, loughborough Leics, LE11, SRG UK (lot 9930226 080). Methanol and acetonitrile (HPLC grade) were purchased from E. Merck, Germany.

II. *Apparatus*

A reverse-phase high-performance liquid chromatograph (Shimadzu, Japan) equipped with a Hypersil C18 column (3.9 mm i.d. × 15 cm, 3 µm, Japan), an UV detector and a recording digital integrator (C-R6A, Shimadzu, Japan) was used. The UV detector was set at 254 nm, and the recorder was set at 1.0 cm/min.

III. *Mobile Phase*

The mobile phase was made of methanol/acetonitrile/0.01 M 1-octane sulfonic acid sodium in 0.005 M phosphate buffer (pH 4.0) (50/50/100). The mobile phase was prepared fresh daily and degassed by a water aspirator immediately prior to use. The flow rate of 1.0 mL/min was used.

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IV. Working Standard Solutions of Calibration Curve

The stock solution was prepared by dissolving 100 mg of morphine HCl, 100 mg of ketamine HCl and 10 mg of droperidol in 0.9% sodium chloride in a 10 mL calibrated volumetric flask and diluting to volume with the mobile phase. Aliquots of 0.5, 1.0, 2.0, and 3.0 mL of the stock standard solution were accurately pipetted into 25 mL calibrated volumetric flasks and diluted to volume with the mobile phase. The solutions were prepared fresh daily for establishing a standard curve.

V. *Validation of the HPLC Method*

A stock solution of morphine HCl admixture was prepared with a concentration of approximately 1.0 mg/mL morphine HCl, 1.0 mg/mL ketamine HCl and 21.4 μg/mL droperidol. Four standard solutions ranging from 0.2-1.2 mg/mL morphine HCl, 0.2-1.2 mg/mL ketamine HCl and 20-120 μg/mL droperidol were prepared by volumetric dilution with mobile phase. Each standard solution was assayed three times. Calibration curves for the mean (SD) active ingredient peak heights against original active ingredient concentrations were constructed. Linear regression analysis was performed to calculate the regression equation and the limit of detection (LOD) from

$$LOD = 3.3\ SD/S \quad (I)$$

Where SD was the standard derivation of the Y-intercept and S was the slope of the calibration curve.

VI. *Determination of Morphine HCl, Ketamine HCl and Droperidol in Admixture Injection*

The mobile phase was pumped through a C18 column that was set at a flow rate of 1.0 mL/min at room temperature. The analytical procedure was initiated after a stable baseline was achieved when the working standard solution was injected. Standard working solutions containing 1.0 mg/mL morphine HCl, 1.0 mg/mL ketamine HCl and 21.4 μg/mL droperidol were injected into to obtain calibration curves. Before running the sample, the percentage recovery of morphine HCl, ketamine HCl and droperidol in the sample solution using the HPLC method might be obtained either by the calibration curve method or by calculations using equation (II) (the mass of sample taken is assumed to be the same as the mass of standard morphine HCl, ketamine HCl and droperidol in a specific working standard solution).

$$\text{Recovery (\%)} = D_{sample}/D_{standard} \times 100\% \quad (II)$$

Where D_{sample} is the peak height of morphine HCL, ketamine HCl or droperidol in the sample solution, and $D_{standard}$ is the peak height of morphine HCl, ketamine HCl or droperidol in the working standard solution.

RESULTS AND DISCUSSION

I. *High Performance Liquid Chromatography (HPLC) Assay*

The HPLC method developed in this study provides excellent simultaneous separation and quantitation of morphine HCl, ketamine HCl and droperidol. The stability-indicating nature of the assay was demonstrated in the chromatograms obtained from solutions forcibly degraded by temperature with either 0.1 mL of 6 N sodium hydroxide adjusted to pH 12 at 60˚C for 6 hr, 0.1 mL of 6N hydrochloric acid adjusted to pH 2 at 60˚C for 6 hr, or 3% hydrogen peroxide at 60˚C for 6 hr, respectively. Reduction in the component peak heights was noted in the chromatograms (Figure 1). The purity of the chromatographic peaks of interest was confirmed by using a photodiode array detector within the wavelength range of 192-360 nm. The relative retention times for morphine HCl, ketamine HCl and droperidol were 6.9, 10.1 and 14.2 min, respectively. The HPLC analytical parameters are listed in Table 1. The HPLC assay showed good separation of the peaks of morphine HCl, ketamine HCl, droperidol and other apparent degradation products (Figure 1) and is indicative of the stability.

II. *Calibration Curves and Reproducibility*

The calibration curves of morphine HCl, ketamine HCl and droperidol were established by plotting the peak heights of each active ingredient against its associated concentrations. The concentration used for each drug was 0.2-1.2 mg/mL for morphine HCl, 0.2-1.2 mg/mL for ketamine HCl and 20-120 μg/mL for droperidol. A statistical linear regression was performed for each agent using concentra-

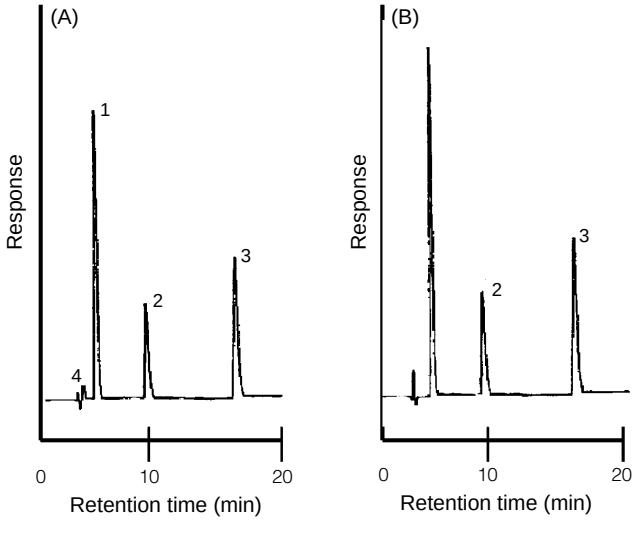

Figure 1. Chromatograms of morphine HCl, ketamine HCl and droperidol as (A) freshly prepared and (B) after exposure to a strong base and beat. Peak 1: morphine HCl; peak 2: ketamine HCl; peak 3: droperidol; peak 4: unidentified degradation products.

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Table 1. The HPLC parameters for the determination of morphine HCl, ketamine-HCl and droperidol

	Morphine	Ketamine	Droperidol
K' (capacity factor)	0.44	1.18	3.67
α (selectivity)	2.68	3.11	
As (asymmetry factor)	1.0	1.0	1.0
Rs (resolution)	6.0	13.3	
N (plate number)	1024/15 cm	1600/15 cm	1806/15 cm

$k = (Ti-Tm)/Tm$, $\alpha_{xy}=k'_y/k'_x$, $\alpha_{yz}=k'_z/k'_y$

k'_x: capacity factor of morphine; k'_y: capacity factor of ketamine; and k'_z: capacity factor of dropendol.

As: as defined in the USP XXII.

Rsxy = (Tiy – Tix)/(1/2Wx + 1/2Wy); Rsyz = (Tiz – Tiy)/(1/2Wz + 1/2Wy)

Ti: retention time of active ingredient; Tm: retention time of mobile phase; Rsxy: resolution value of morphine HCl and ketamine HCl; Rsyz: resolution value of morphine HCl and droperidol. Tix: retention time of morphine HCl peak; Tiy: retention time of ketamine HCl peak; Tiz: retention time droperidol peak. 1/2Wx: half wave width of morphine HCl peak; 1/2Wy: half wave width of ketamine HCl peak; 1/2Wz: half wave width of droperidol peak

$N = 16 (Ti/wi)^2$, wave width of each compound peak.

Table 2. Assay of morphine HCl, ketamine HCl and droperidol injections

Preparation	Labelled amount	% Recovery[a]		
	(mg/mL)	Mean	S D	C V
A[b]	10	103.2	1.16	1.12
B[c]	50	101.4	1.34	1.32
C[d]	2.5	101.0	0.50	0.50
D[+e] contains				
Morphine HCl	1.0	103.2	1.38	1.34
Ketamine HCl	1.0	101.9	1.66	1.63
Droperidol	0.0241	101.1	0.25	0.25

[a]Results of three replicate determinations: SD = standard deviation; CV = coefficient of variation.

[b]10 mL of Morphine HCl injection, 10 mg/mL, National Bureau of Controlled Drugs, Executive Yuan, Taiwan, ROC (lot 890805).

[c]Ketalar®, 50mg/mL, 10 mL. pake-David, USA (lot ZH708).

[d]10 mL of Dehyobenzperidol®, 2.5 mg/mL, Janssen Pharmaceutic, USA, (lot EB314).

[e]I.V. admixtures of morphine HCl, ketamine HCl and droperidol in 0.9% sodium chloride were prepared by commercially available products.

tion against peak height. Three regression lines (Y = aX + b; Y = peak height, X = concentration) were obtained for morphine HCl, a = 6.5343 ± 0.004199, b = 0.02957 ± 0.001151, r = 0.9999; for ketamine HCl, a = 1.0924 ± 0.001019, b = 0.01387 ± 0.0000864, r = 0.9995; for droperidol, a = 1.1624 ± 0.006223, b = 0.04914 ± 0.0006946, r = 0.9999. The limit of detection (LOD) for morphine HCl, ketamine HCl and droperidol were 15.73 μg/mL, 0.2610 μg/mL and 1.802 μg/mL, respectively. Inter-day and intra-day variations for morphine HCl, ketamine HCl and droperidol in the same concentration ranges were determined to be 2.25%, 2.33%, 3.42% and 3.66%, 2.12% and 3.08%, respectivity. The utility of the method commercially available injections (Table 2).

CONCLUSIONS

This study successfully concluded the development of a HPLC method to simultaneously determine the concentrations of morphine HCl, ketamine HCl and droperidol from their I.V. admixtures in 0.9% sodium chloride solution. This method is simple, rapid, precise and indicative of the stability.

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